

# PATENT COOPERATION TREATY

**PCT**

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner  
US Department of Commerce  
United States Patent and Trademark  
Office, PCT  
2011 South Clark Place Room  
CP2/5C24  
Arlington, VA 22202  
ETATS-UNIS D'AMERIQUE  
in its capacity as elected Office

<b>Date of mailing</b> (day/month/year) 17 July 2001 (17.07.01)	
<b>International application No.</b> PCT/US00/27210	<b>Applicant's or agent's file reference</b> 2847-56232
<b>International filing date</b> (day/month/year) 02 October 2000 (02.10.00)	<b>Priority date</b> (day/month/year) 01 October 1999 (01.10.99)
<b>Applicant</b> HINTZ, William, E. et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:  
27 April 2001 (27.04.01)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was  
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

<b>The International Bureau of WIPO</b> 34, chemin des Colombettes 1211 Geneva 20, Switzerland	<b>Authorized officer</b> Odile ALIU
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

# PATENT COOPERATION TREATY

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

## PCT

### WRITTEN OPINION

(PCT Rule 66)

To: **PAULA A. DE GRANDIS**  
**KLARQUIST, SPARKMAN, CAMPBELL, LEIGH &**  
**WHINSTON, LLP**  
**ONE WORLD TRADE CENTER, SUITE 1600**  
**121 SW SALMON STREET**  
**PORTLAND, OR 97204**

SLR

Date of Mailing  
(day/month/year)

17 AUG 2001

Applicant's or agent's file reference  
2847-56232

**REPLY DUE**  
within **TWO** months  
from the above date of mailing

International application No.  
PCT/US00/27210

International filing date (day/month/year)  
02 OCTOBER 2000

Priority date (day/month/year)  
01 OCTOBER 1999

International Patent Classification (IPC) or both national and  
IPC(7): C12N 9/00, 9/24, 15/09, 1/20; C07K, 16/00 a

IPC  
B5/69.2, 183, 200; 530/389.1

Applicant  
UNIVERSITY OF VICTORIA INNOVATION AND DEVELOPMENT CORPORATION

1. This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- |      |                                     |  |   |
|------|-------------------------------------|--|---|
| I    | <input checked="" type="checkbox"/> | Basis of the opinion   | DOCKETED FOR: <u>10/17/01</u><br><br>COMPUTER <input checked="" type="checkbox"/><br>CARD <input type="checkbox"/><br>BOOK <input checked="" type="checkbox"/><br>DRAWER <input checked="" type="checkbox"/><br>BKPR <input type="checkbox"/><br>ANN SVF <input type="checkbox"/> |
| II   | <input type="checkbox"/>            | Priority   |   |
| III  | <input type="checkbox"/>            | Non-establishment of opinion with regard to novelty, inventive step or industrial applicability  |   |
| IV   | <input type="checkbox"/>            | Lack of unity of invention   |   |
| V    | <input checked="" type="checkbox"/> | Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |   |
| VI   | <input type="checkbox"/>            | Certain documents cited  |   |
| VII  | <input type="checkbox"/>            | Certain defects in the international application   |   |
| VIII | <input type="checkbox"/>            | Certain observations on the international application  |   |

3. The applicant is hereby invited to reply to this opinion.

**When?** See the time limit indicated above. ~~The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).~~

**How?** By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

**Also** For an additional opportunity to submit amendments, see Rule 66.4.  
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 *bis*.  
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 01 FEBRUARY 2002

Name and mailing address of the IPEA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

**TERRY J. DEY**

MANJUNATH RAO

**PARALEGAL SPECIALIST**  
**TECHNOLOGY CENTER 1600**

Telephone No. (703) 308-0196

WRITTEN OPINION

International application No.

PCT/US00/27210

**I. Basis of the opinion**

1. With regard to the elements of the international application:\*

☒ the international application as originally filed

☒ the description:

pages 1-44 , as originally filed  
pages NONE , filed with the demand  
pages NONE , filed with the letter of

☒ the claims:

pages 45-47 , as originally filed  
pages NONE , as amended (together with any statement) under Article 19  
pages NONE , filed with the demand  
pages NONE , filed with the letter of

☒ the drawings:

pages 1-22 , as originally filed  
pages NONE , filed with the demand  
pages NONE , filed with the letter of

☒ the sequence listing part of the description:

pages 1-13 , as originally filed  
pages NONE , filed with the demand  
pages NONE , filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language \_\_\_\_\_ which is:

- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).  
☐ the language of publication of the international application (under Rule 48.3(b)).  
☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the written opinion was drawn on the basis of the sequence listing:

- ☒ contained in the international application in printed form.  
☒ filed together with the international application in computer readable form.  
☐ furnished subsequently to this Authority in written form.  
☐ furnished subsequently to this Authority in computer readable form.  
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

- ☒ the description, pages NONE  
☒ the claims, Nos. NONE  
☒ the drawings, sheets/fig NONE

5. ☐ This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".

**V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement****1. statement**

Novelty (N)	Claims <u>2, 7</u>	YES
	Claims <u>1, 3-6, 8-14</u>	NO
Inventive Step (IS)	Claims <u>NONE</u>	YES
	Claims <u>1-14</u>	NO
Industrial Applicability (IA)	Claims <u>NONE</u>	YES
	Claims <u>NONE</u>	NO

**2. citations and explanations**

Claims 1, 3-6, 8-14 lack novelty under PCT Article 33(2) as being anticipated by Inoue et al.

Claims 1, 3-6, 8-14 are drawn to a purified protein having mannosidase activity comprising an amino acid sequence selected from the group consisting of a) SEQ ID NOs: 3, 6, and 18, b) a conservative variant, or c) amino acid sequences having at least 60% sequence identity to the sequences specified in a) (claim 1), an isolated nucleic acid encoding the same, vector, host cell, a method for altering glycosylation pattern of a macromolecule (claims 3-6, 8-10), an isolated nucleic acid comprising a sequence selected from the group consisting of at least 15 nucleotides of SEQ ID NO: 1 (claim 11(a)), a method for altering glycosylation pattern (claim 12), a method for isolating nucleic acid sequence encoding a mannosidase and a mannosidase identified by the above method (claims 13-14). Inoue et al. disclose a the isolation and identification of a mannosidase that is more than 60% identical to SEQ ID NO: 6 and also disclose a nucleic acid encoding a mannosidase which is at least 15 nucleotides identical to SEQ ID NO: 1, and the above described methods. Therefore Inoue et al. anticipate claims 1, 3-6, 8-14 of the instant application as written.

Claim 11 lacks novelty under PCT Article 33(2) as being anticipated by Kupfer et al.

Claim 11 is drawn to an isolated nucleic acid molecule comprising a sequence selected from a group consisting of at least 15, 20, 30 nucleotides of SEQ ID NO: 1, 4 or 17. Kupfer et al. disclose a nucleic acid molecule comprising 15, 20 or 30 nucleotides of SEQ ID NO: 17. Therefore Kupfer et al. anticipate claim 11 of the instant application as written.

Claims 1-14 lack an inventive step under PCT Article 33(3) as being obvious over Inoue et al. in view of Turco et al. Claims 1-14 are drawn to a purified protein having mannosidase activity comprising an amino acid sequence selected from the group consisting of a) SEQ ID NOs: 3, 6, and 18, b) a conservative (Continued on Supplemental Sheet.)

**Supplemental Box**

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

**TIME LIMIT:**

The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

**V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):**

variant, or c) amino acid sequences having at least 60% sequence identity to the sequences specified in a) (claim 1), a binding agent (claim 2), an isolated nucleic acid encoding the same, vector, host cell, a method for altering glycosylation pattern of a macromolecule (claims 3-6, 8-10), a transgenic fungus (claim 7), an isolated nucleic acid comprising a sequence selected from the group consisting of at least 15 nucleotides of SEQ ID NO:1 (claim 11(a)), a method for altering glycosylation pattern (claim 12), a method for isolating nucleic acid sequence encoding a mannosidase and a mannosidase identified by the above method (claims 13-14). Inoue et al. disclose a mannosidase that more than 60% identical to SEQ ID NO:6 and also a nucleic acid sequence encoding such a mannosidase and wherein at least 15 nucleotides are identical to SEQ ID NO:1. However, the reference does not teach the production of a transgenic fungus or the glycosylation methods. Turco et al. teach the different methods of glycosylation using a mannosidase enzyme. It would have been obvious to one of ordinary skill in the art to combine the above two references with the high level of knowledge in the art of molecular biology to make a transgenic fungus comprising the above alpha-mannosidase and also to develop an assay to detect other mannosidases using the above polynucleotide sequences. One of ordinary skill in the art would have been motivated to do so as Inoue et al. provide a new alpha-mannosidase isolated from a industrially important fungus. One of skill in the art would have had a reasonable expectation of success since Inoue et al provide methods for all the above and also provide a nucleic acid sequence and an amino acid sequence of a alpha-mannosidase. therefore, the above invention would have been *prima facie* obvious to one of ordinary skill in the art.

Claims 2 and 7 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest a binding agent or a transgenic fungus for alpha-mannosidase.

**----- NEW CITATIONS -----**

Database GenBank Accession No. AA787675, (KUPFER et al.) An *Aspergillus nidulans* EST database, 31 July 1998.  
Database Sptrembl\_16 Accession No. Q12563, (INOUE et al.) Alpha mannosidase, 01 November 1996.

# PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

## PCT

### NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

**To:** PAULA A. DE GRANDIS  
KLARQUIST, SPARKMAN, CAMPBELL, LEIGH &  
WHINSTON, LLP  
ONE WORLD TRADE CENTER, SUITE 1600  
121 SW SALMON STREET  
PORTLAND, OR 97204

Date of Mailing  
(day/month/year) **28 FEB 2001**

Applicant's or agent's file reference  
**2847-56232**

**FOR FURTHER ACTION See paragraphs 1 and 4 below**

International application No.  
**PCT/US00/27210**

International filing date  
(day/month/year)  
**02 OCTOBER 2000**

Applicant  
**UNIVERSITY OF VICTORIA INNOVATION AND DEVELOPMENT CORPORATION**

1. ☒ The applicant is hereby notified that the international search report has been established and is transmitted herewith.

**Filing of amendments and statement under Article 19:**

The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

**When?** The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

**Where?** Directly to the International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland  
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 *bis* 1 and 90 *bis* 3, respectively, before the completion of the technical preparations for international publication.

Within 19 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

MANJUNATH RAO

Telephone No. (703) 305-3230

**TERRY J. DEY**  
**PARALEGAL SPECIALIST**  
**TECHNOLOGY CENTER 1600**

# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 2847-56232	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/US00/27210	International filing date ( <i>day/month/year</i> ) 02 OCTOBER 2000	(Earliest) Priority Date ( <i>day/month/year</i> ) 01 OCTOBER 1999
Applicant UNIVERSITY OF VICTORIA INNOVATION AND DEVELOPMENT CORPORATION		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

**1. Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☒ Unity of invention is lacking (See Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. \_\_\_\_\_

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/27210

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/27210

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 9/00, 9/24, 15/09, 1/20; C07K 16/00

US CL : 435/69.2, 183, 200; 530/389.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.2, 183, 200; 530/389.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, CAPLUS, EMBASE, MEDLINE, SCISEARCH, USPTO WEST.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	HERSCOVIC ET AL. Isolation of a mouse golgi mannosidase cDNA, a member of a gene family conserved from yeasts to mammals. J. Biol. Chem. 01 April 1994, Vol. 269, No. 13, pages 9864-9871, see especially pages 9870-9871.	1, 3-14 ----- 1, 3-14
X --- Y	LAL ET AL. Isolation and expression of murine and rabbit cDNAs encoding an alpha-mannosidase involved in the processing of asparagine-linked oligosaccharides. J. Biol. Chem. 01 April 1994, Vol. 269, No. 13, pages 9872-9881, see entire article.	1, 3-14 ----- 1, 3-14
Y	TURCO ET AL. Altered G-protein glycosylation in vesicular stomatitis virus-infected glucose-deprived baby hamster kidney cells. J. Biol. Chem. 10 August 1982, Vol. 257, No. 15, pages 8674-8679, see entire article.	1, 3-14

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* & * document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

15 JANUARY 2001

Date of mailing of the international search report

28 FEB 2001

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

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TERRY J. DEY  
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TECHNOLOGY CENTER 1600

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/27210

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,837,836 A (FRIDERICI ET AL.) 17 November 1998 (17/11/98) see entire document.	2 --- 2
X --- Y	US 5,578,484 A (HOROSZEWICZ) 26 November 1996 (26/11/96) see entire document.	2 --- 2
X --- Y	EADES ET AL. Identification and analysis of a class 2 alpha-mannosidase from Aspergillus nidulans. Glycobiology. 1998, Vol. 8, pages 17-33, see entire document.	1-14 --- 1-14
X --- Y	INOUE ET AL. Molecular cloning and nucleotide sequence of the 1,2-alpha-D-mannosidase gene, msds, from Aspergillus saitoi and expression of the gene in yeast cells. Biochimica et Biophysica Acta. 1995, Vol. 1253, pages 141-145, see entire document.	1-14 --- 1-14

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING**

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1, 3-7, 11, 13-14, drawn to a purified mannosidase, polynucleotides and method of making.

Group II, claim 2, drawn to a binding agent that binds the purified protein.

Group III, claims 8-10 and 12, drawn to a method of altering glycosylation pattern of a macromolecule.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Mannosidases, their polynucleotides, binding agents and method of altering the glycosylation pattern of macromolecules are well known in the prior art. Thus, the above invention, when considered as a whole does not contribute over the prior art (see Turco et al. J. Biol. Chem., 1982, Vol. 257(15):8674-8679).

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
12 April 2001 (12.04.2001)

PCT

(10) International Publication Number  
**WO 01/25406 A1**

(51) International Patent Classification<sup>7</sup>: C12N 9/00,  
9/24, 15/09, 1/20, C07K 16/00

(21) International Application Number: PCT/US00/27210

(22) International Filing Date: 2 October 2000 (02.10.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/157,341 1 October 1999 (01.10.1999) US

(71) Applicant (for all designated States except US): UNI-  
VERSITY OF VICTORIA INNOVATION & DEVELOPMENT CORPORATION [CA/CA]; Box 3075 R-Hut,  
McKenzie Avenue, Victoria, British Columbia V8W 3W2 (CA).

(72) Inventors; and

(75) Inventors/Applicants (for US only): HINTZ, William, E.  
[CA/CA]; 5601 Matterhorn Court, Victoria, British Columbia V9E 2G1 (CA). EADES, Caleb, Joshua [US/CA]; 852  
Leslie Drive, Victoria, British Columbia V8X 2Y4 (CA).

(74) Agent: DE GRANDIS, Paula, A.; Klarquist, Sparkman,  
Campbell, Leigh & Winston, LLP, Suite 1600, One World  
Trade Center, 121 S.W. Salmon Street, Portland, OR 97204 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**

- With international search report.
- Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MANNOSIDASES AND METHODS FOR USING SAME

(57) Abstract: Mannosidase enzymes and use of such enzymes to alter the glycosylation patterns of macromolecules are disclosed. Also disclosed are the nucleic acid sequences encoding the mannosidase enzymes.

WO 01/25406 A1

10 REC'D 25 MAR 2002

## SEQUENCE LISTING

&lt;110&gt; Hintz, William et al.

&lt;120&gt; Mannosidases and Methods for using the Same

&lt;130&gt; 56232

&lt;140&gt;

&lt;141&gt;

&lt;150&gt; 60/157,341

&lt;151&gt; 1999-10-01

&lt;160&gt; 19

&lt;170&gt; PatentIn Ver. 2.0

&lt;210&gt; 1

&lt;211&gt; 3328

&lt;212&gt; DNA

&lt;213&gt; Aspergillus nidulans

&lt;400&gt; 1

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 <213> *Aspergillus nidulans*

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```

```

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 <212> PRT  
 <213> Aspergillus nidulans

<400> 3

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```

```

Phe Val Leu Leu Ile Phe His Phe Ser Arg Leu Ala Val Thr Ile Ser
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```

```

Leu Gln Ser Trp Val Pro Pro Pro Val Asp His His Asn Pro Pro
      35             40             45

```

```

Phe Pro Asp Gln Asn Leu Lys Asp Pro Tyr Glu Asn Asp Asn Ser Ala
      50             55             60

```

```

Thr Gly Ser Gly Ala Pro Pro Pro Ala Leu Val Glu Pro Glu Glu Tyr
      65             70             75             80

```

```

Gln Arg Pro Pro Leu Tyr Thr Asp Ser Asp Asp Ser Pro Thr Pro Ser
      85             90             95

```

```

Lys Glu Arg Leu Asp Thr Pro Ser Asn Val Pro Ser Gln Glu Pro Glu
      100            105            110

```

```

Phe Asp Ala Ala Arg Leu Gln Thr Gly Ala Gln Thr Gln Asn Lys His
      115            120            125

```

```

Glu Asp Asp Glu Asp Ile Val Pro Ile Ser His Trp Lys Pro Met Pro
      130            135            140

```

```

Glu Arg His Pro Val Ser Pro Glu Ala Leu Ile Lys Leu Pro Thr Gly
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```

```

Gln Ser Lys Glu Leu Pro Gln Leu Gln Ala Lys Phe Lys Asp Glu Ser
      165            170            175

```

```

Ser Ser Asp Lys Met Gln Arg Leu Gln Gln Leu Asp Thr Ile Lys Ser
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```

```

Ala Phe Leu His Ala Trp Asn Gly Tyr Lys Ile Ser Ala Met Gly His
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```

```

Asp Glu Val Arg Pro Leu Arg Gly Gly Phe Lys Asp Thr Phe Asn Gly
      210            215            220

```

```

Trp Gly Ala Thr Leu Val Asp Ala Leu Asp Thr Leu Trp Ile Met Asp
      225            230            235            240

```

```

Leu Lys Glu Glu Phe Ser Met Ala Val Asp Tyr Val Lys Lys Ile Asp
      245            250            255

```

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 290 295 300  
 Asp Ala Phe Asp Thr Pro Asn Arg Met Pro Thr Leu Tyr Tyr Lys Trp  
 305 310 315 320  
 Ser Pro Glu Tyr Ala Ser Glu Phe Arg Arg Gly Asp Phe Lys Ala Val  
 325 330 335  
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 Glu Leu Glu Lys Tyr Gln Asp Leu Thr Lys Leu Pro Gly Leu Trp Pro  
 370 375 380  
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 Glu Pro Ala Ala Ala Gly Gln Pro Val Arg Trp Ser Ser Asp Glu Ile  
 405 410 415  
 Asn Ser Thr Ser Ser Val Ser Tyr Arg Thr Arg Gln Ile His Glu Gly  
 420 425 430  
 Gly Glu Pro Val Arg His Asp Asn Asp Ser Phe Glu Thr Gly Phe Pro  
 435 440 445  
 Val Ser Val Asp Thr Arg Thr Pro Pro Pro Lys Gln Asp Cys Thr Gly  
 450 455 460  
 Gly Leu Asn Asp Gln Leu Ser Gly Ile Asp Lys Phe Gly Leu Gly Ala  
 465 470 475 480  
 Leu Gly Asp Ser Thr Tyr Glu Tyr Leu Pro Lys Glu Tyr Met Leu Leu  
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 Gly Gly Asn Asn Asp Gln Tyr Leu Asn Met Tyr Gln Lys Ala Met Asp  
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 Thr Val Arg Glu Tyr Leu Val Tyr Gln Pro Met Leu Lys Asn Asn Arg  
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 Asp Val Arg Phe Leu Ala Thr Val Ser Met Thr Lys Ser Leu Asp Ala  
 530 535 540  
 Asn Pro Pro Gly Arg Thr Phe Ala Tyr Glu Gly Thr His Leu Thr  
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 Cys Phe Ala Gly Gly Met Leu Ala Ile Gly Ala Lys Leu Phe Gly Leu  
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Asp Lys Asp Leu Lys Leu Gly Ser Gln Leu Thr Asp Gly Cys Val Trp  
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 595 600 605  
 Val Pro Cys Lys Lys Gly Glu Pro Cys Glu Trp Asp Glu Asp Ala Tyr  
 610 615 620  
 Tyr Met Ala Met Asp Pro Tyr Ala Asp Lys Arg Pro Ile Ser His Asn  
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 Lys Arg Ser Ala Gly Pro Glu Lys Gly Asn Trp His Val Val Ala Thr  
 645 650 655  
 Ala Glu Ser Ser Ser Pro Gln Glu Asp Lys Thr Gln Lys Ser Thr Thr  
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 675 680 685  
 His Glu Glu Phe Val Thr Gly Lys Ile Leu Asn Asp Arg Leu Pro Pro  
 690 695 700  
 Gly Met Thr Gly Ile Ser Ala Arg Gln Tyr Leu Leu Arg Pro Glu Ala  
 705 710 715 720  
 Ile Glu Ser Val Phe Ile Met Phe Arg Leu Thr Gly Asp Pro Ser Trp  
 725 730 735  
 Arg Glu Lys Gly Trp Lys Met Phe Gln Ala Val Asp Lys Ala Thr Lys  
 740 745 750  
 Thr Glu Leu Ala Asn Ser Ala Ile Ser Asp Val Thr Val Asp Asn Pro  
 755 760 765  
 Arg Pro Val Asp Ser Met Glu Ser Phe Trp Leu Ala Glu Thr Leu Lys  
 770 775 780  
 Tyr Phe Tyr Leu Leu Phe Ser Asp Pro Ser Leu Val Ser Leu Glu Glu  
 785 790 795 800  
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&lt;210&gt; 4

&lt;211&gt; 2177

&lt;212&gt; DNA

<213> *Aspergillus nidulans*

&lt;400&gt; 4

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&lt;210&gt; 5

&lt;211&gt; 1515

&lt;212&gt; DNA

<213> *Aspergillus nidulans*

&lt;400&gt; 5

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 <212> PRT  
 <213> Aspergillus nidulans

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 Arg Ala Asp Ala Val Lys Glu Ala Phe Ser His Ala Trp Asp Gly Tyr  
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 Tyr Asn Tyr Ala Phe Pro His Asp Glu Leu His Pro Ile Ser Asn Gly  
           50                    55                    60  
 Tyr Gly Asp Ser Arg Asn His Trp Gly Ala Ser Ala Val Asp Ala Leu  
           65                    70                    75                    80  
 Ser Thr Ala Ile Met Met Arg Asn Ala Thr Ile Val Asn Gln Ile Leu  
                     85                    90                    95  
 Asp His Ile Ala Ala Val Asp Tyr Ser Lys Thr Asn Ala Met Val Ser  
           100                    105                    110  
 Leu Phe Glu Thr Thr Ile Arg Tyr Leu Ala Gly Met Ile Ser Gly Tyr  
           115                    120                    125  
 Asp Leu Leu Lys Gly Pro Ala Ala Gly Leu Val Asp Asp Ser Arg Val  
           130                    135                    140  
 Asp Val Leu Leu Glu Gln Ser Gln Asn Leu Ala Glu Val Leu Lys Phe  
           145                    150                    155                    160  
 Ala Phe Asp Thr Pro Ser Gly Val Pro Tyr Asn Met Ile Asn Ile Thr  
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 Ser Gly Gly Asn Asp Gly Ala Thr Thr Asn Gly Leu Ala Val Thr Gly  
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           210                    215                    220  
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 Asn Ile Ala Asp Gly Lys Leu Ala Asn Gly His Ile Ser Trp Asn Gly  
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 Gly Ala Asp Ser Tyr Tyr Glu Tyr Leu Ile Lys Met Tyr Val Tyr Asp  
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 Pro Glu Arg Phe Gly Leu Tyr Arg Asp Arg Trp Val Ala Ala Ala Glu  
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 Ile Cys Pro Ser Thr Pro Pro Gln Pro Pro Tyr Asn Arg Thr Ser Thr  
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 65 70 75 80  
 Phe Ile Pro Leu Ser Thr Asn Ser Pro Ala Thr Leu Pro Arg Ile Gln  
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 Arg Ser Ser Phe Pro Leu Gln Ser Ser Ile Thr Lys Ser Arg Gln Ala  
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 Ala Val Lys Gly Ala Phe Gln Arg Ala Trp Thr Ser Tyr Thr Thr His  
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 Pro Gln Arg Ala Ala Val Gln Gly Val Leu Ala Glu Leu Ala Ser Ser  
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 Ser Leu Glu Phe Thr Arg Leu Ser Gln Leu Thr Gly Asp Met Arg Tyr  
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 His Thr Arg Ile Pro Gly Leu Trp Pro Val Ser Val Asn Leu Gln Lys  
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 Gly Asp Leu Thr Arg Gly Ser Thr Phe Ser Phe Gly Gly Met Ala Asp

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Ser	Ala	Tyr	Glu 340	Tyr	Leu	Gly	Lys	Thr 345	Tyr	Arg	Leu	Leu	Gly 350	Gly	Val
Gly	Lys	Gly 355	Pro	Gln	Tyr	Glu	Arg 360	Leu	Ala	Arg	Asn	Ala 365	Leu	Asp	Ala
Gly	Ile 370	Arg	His	Leu	Leu	Phe 375	Arg	Pro	Met	Thr	Pro 380	Asp	His	Ala	Asp
Ile 385	Leu	Leu	Pro	Gly	Val 390	Ala	His	Ala	Thr	Ser 395	Ser	Ser	Val	Gly	Leu 400
Glu	Pro	Arg	Thr 405	Glu	His	Leu	Ala	Cys	Phe 410	Val	Gly	Gly	Met	Tyr	Ala
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Lys	Leu	Thr 435	Asp	Gly	Cys	Ile	Trp 440	Tyr	Tyr	Asp	Asn	Ser 445	Pro	Leu	Gly
Ile 450	Met	Pro	Glu	Met	Phe 455	Thr	Val	Pro	Ala	Cys	Pro 460	Ser	Val	Ala	Glu
Cys 465	Pro	Trp	Asp	Glu	Thr 470	Arg	Gly	Gly	Ile	Tyr 475	Thr	Tyr	Val	Arg	Asp 480
Gly	His	Tyr	Phe 485	Leu	Arg	Pro	Glu	Ala 490	Met	Glu	Ser	Ile	Phe 495	Tyr	Met
Trp	Arg	Ile 500	Thr	Gly	Asp	Glu	Lys	Tyr 505	Arg	Glu	Ala	Ala	Trp 510	Arg	Met
Phe	Thr 515	Ala	Ile	Glu	Ala	Val	Thr 520	Lys	Thr	Glu	Phe 525	Gly	Asn	Ala	Ala
Val 530	Arg	Asp	Val	Met	Val	Glu 535	Glu	Gly	Asn	Val	Lys 540	Arg	Glu	Asp	Ser
Met 545	Glu	Ser	Phe	Trp	Met 550	Ala	Glu	Thr	Leu	Lys 555	Tyr	Leu	Tyr	Leu	Ile 560
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<223> Description of Artificial Sequence: CONSENSUS  
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6

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/27210

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 9/00, 9/24, 15/09, 1/20; C07K 16/00  
US CL : 435/69.2, 183, 200; 530/389.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.2, 183, 200; 530/389.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, CAPLUS, EMBASE, MEDLINE, SCISEARCH, USPTO WEST.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	HERSCOVIC ET AL. Isolation of a mouse golgi mannosidase cDNA, a member of a gene family conserved from yeasts to mammals. J. Biol. Chem. 01 April 1994, Vol. 269, No. 13, pages 9864-9871, see especially pages 9870-9871.	1, 3-14 ----- 1, 3-14
X --- Y	LAL ET AL. Isolation and expression of murine and rabbit cDNAs encoding an alpha-mannosidase involved in the processing of asparagine-linked oligosaccharides. J. Biol. Chem. 01 April 1994, Vol. 269, No. 13, pages 9872-9881, see entire article.	1, 3-14 ----- 1, 3-14
Y	TURCO ET AL. Altered G-protein glycosylation in vesicular stomatitis virus-infected glucose-deprived baby hamster kidney cells. J. Biol. Chem. 10 August 1982, Vol. 257, No. 15, pages 8674-8679, see entire article.	1, 3-14



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	* T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A* document defining the general state of the art which is not considered to be of particular relevance	* X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
* E* earlier document published on or after the international filing date	* Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
* L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	* &* document member of the same patent family
* O* document referring to an oral disclosure, use, exhibition or other means	
* P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

15 JANUARY 2001

Date of mailing of the international search report

28 FEB 2001

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
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**PARALEGAL SPECIALIST**  
**TECHNOLOGY CENTER 1600**

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/27210

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,837,836 A (FRIDERICI ET AL.) 17 November 1998 (17/11/98) see entire document.	2 --- 2
X --- Y	US 5,578,484 A (HOROSZEWICZ) 26 November 1996 (26/11/96) see entire document.	2 --- 2
X --- Y	EADES ET AL. Identification and analysis of a class 2 alpha-mannosidase from <i>Aspergillus nidulans</i> . <i>Glycobiology</i> . 1998, Vol. 8, pages 17-33, see entire document.	1-14 --- 1-14
X --- Y	INOUE ET AL. Molecular cloning and nucleotide sequence of the 1,2-alpha-D-mannosidase gene, msds, from <i>Aspergillus saitoi</i> and expression of the gene in yeast cells. <i>Biochimica et Biophysica Acta</i> . 1995, Vol. 1253, pages 141-145, see entire document.	1-14 --- 1-14

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/27210

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/27210

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1, 3-7, 11, 13-14, drawn to a purified mannosidase, polynucleotides and method of making.

Group II, claim 2, drawn to a binding agent that binds the purified protein.

Group III, claims 8-10 and 12, drawn to a method of altering glycosylation pattern of a macromolecule.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Mannosidases, their polynucleotides, binding agents and method of altering the glycosylation pattern of macromolecules are well known in the prior art. Thus, the above invention, when considered as a whole does not contribute over the prior art (see Turco et al. J. Biol. Chem., 1982, Vol. 257(15):8674-8679).